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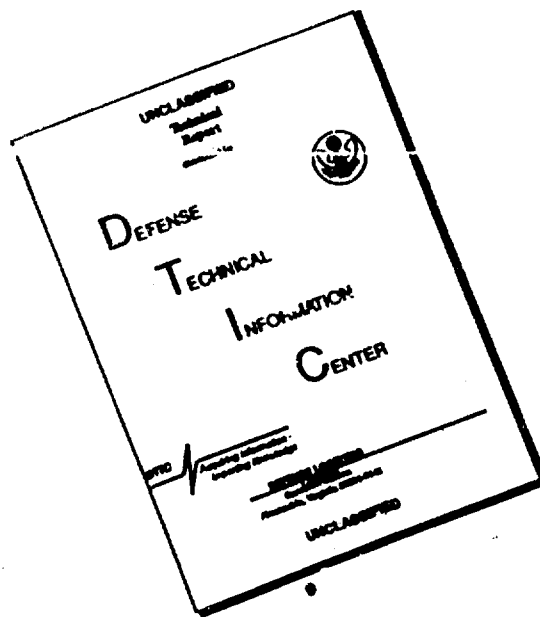
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ADSORPTION OF IMMUNE ANTIBODIES ON PARTICLES OF SUSPENSIONS

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Immunobiologii  
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The study of patterns of adsorption of immune antibodies has established that they are adsorbed by organic compounds containing hydroxyl, quinone, carboxyl, aldehyde, sulfo and nitro groups, by organic compounds the molecules of which include atoms of metals, and also by porous substances (diatomaceous earth, kaolin, activated carbon, and others). The antibodies adsorbed on suspension particles retain their activity and specificity. Suspensions the particles of which contain adsorbed antibodies may be used for accelerated detection and identification of pathogenic bacteria (Adamov, 1956-1962). Our findings on the immunological activity of antibodies adsorbed on solid substances were confirmed by Yafayev (1962-1963) in experiments with activated carbon. Alizarin was found to be best suitable for immunological reactions (Adamov, 1960-1962).

In the present article we submit the results of further investigation of the distinctions of adsorption of immune antibodies and of the properties of suspensions of various substances. This study was performed using the Adamov method which was published in 1960 in Byulleten' Eksperimental'noy biologii i meditsiny (Bulletin of Experimental Biology and Medicine).\* In the experiments we used typhoid [*Salmonella typhosa*] agglutinogenic unadulterated rabbit serum in a titer of 1:25600, and the same serum adsorbed by heterologous bacterial antigens (for the purpose of clearing unstable protein fractions containing no antibodies) the titer

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\*Some of the studies were conducted at the Laboratory of Biochemistry and Biophysics of Bacteria (headed by A.B. Gabrilovich, Candidate of Medical Sciences), Rostov-na-Donu Institute of Epidemiology and Microbiology.

of which constituted 1:6400 following adsorption. Each experiment was repeated at least three times.

The antibody titers were expressed in AE [agglutinating units] in calculating the adsorption activity of different substances. The maximum dilution of serum in which distinct agglutination was observed (++) was considered as one AE. The quantity of antibodies adsorbed per 100 mg [milligrams] of substance was determined using the following equation:

$$\frac{X}{m} = (C - C_1) Y \quad \text{where}$$

$\frac{X}{m}$  is the quantity of antibodies adsorbed per 100 mg of substance,  
C is the concentration of antibodies (in AE) prior to adsorption of serum,  
C<sub>1</sub> is the concentration of antibodies (in AE) following adsorption of serum,  
Y is the volume of adsorbed serum.

Organic substances that had no functional groups and those whose molecules included atoms of chloride did not adsorb immune antibodies (Table 1). Substances that contained hydroxyl groups adsorbed antibodies from unadulterated immune serum and from serum submitted to adsorption by heterologous antigens. The substances whose molecules contained amino and imino groups manifested different adsorption activity. Thus, the anion exchange resin, AN-1, did not adsorb antibodies, while greenish-blue phthalocyaninic (which also contained imino groups) actively adsorbed antibodies from immune sera. Evidently the adsorption of antibodies by agents containing imino groups was determined to a significant extent by their specific arrangement in the molecules. Benzidine adsorbed agglutinins only from serum first submitted to adsorption by heterologous antigens.

In order to investigate the distinctions of antibody adsorption by benzidine (a substance containing amino groups) using the method of paper electrophoresis we made a study of unadulterated typhoid agglutino-genic serum and typhoid serum adsorbed by heterologous antigens. Following adsorption by heterologous antigens there remained a small amount of albumins and globulins, primarily  $\beta$ -fractions in the serum (see Figure). These findings indicated that benzidine adsorbed immune antibodies only from serum containing antibodies and a small amount of other protein fractions.

The significant difference between magnitude of adsorption of antibodies from unadulterated serum and serum adsorbed by heterologous antigens was attributed to the difference in titers of these sera. As revealed by our studies (Adamov, 1959), the magnitude of antibody adsorption by solid agents is proportional to the concentration of antibodies in the serum.

**Table 1**  
**Adsorption activity of organic substances with respect to**  
**typhoid N-agglutinins**

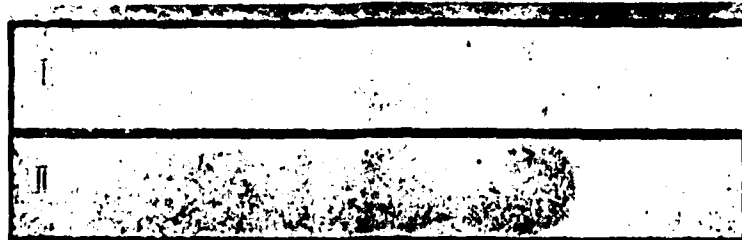
| a)<br>Адсорбент  | b)<br>Функциональ-<br>ная группа | в)<br>Количество АЕ брюшнотифозных N-агглютининов,<br>адсорбированных на 100 мг адсорбента |  |
|--|----------------------------------|--|--|
|  |                                  | г)<br>нестандартная агглютинирующая<br>сысывотка, титр 25600 АЕ                            | е)<br>брюшнотифозная агглю-<br>тинирующая сысывотка,<br>поддерживавшаяся адсорб-<br>цией гетерологичными<br>антигенами, титр 6400 АЕ |
| f) Нафталин . . . . .  | —                                | 0  | 0  |
| g) Антрацен . . . . .  | —                                | 0  | 0  |
| h) Алizarин . . . . .  | —OH                              | 384  | 121,3  |
| i) Антрагаллол . . . . .   | =O                               |  |  |
|  | —OH                              | 384  | 120  |
| j) Индантрон . . . . .   | =O                               | 502  | 124  |
| k) Гексахлорциклогексан . . . . .  | =NH                              |  |  |
| l) Бензидин (основной) . . . . .   | —Cl                              | 0  | 0  |
| m) Аммонит АН-1 . . . . .  | —NH <sub>2</sub>                 | 0  | 96   |
|  | =N                               | 0  | 0  |
|  | =NH                              |  |  |
| n) Пигмент зеленовато-голубой<br>фталоцианиновый (гелиоген<br>голубой Г) . . . . . | =NH                              | 508  | 128  |
|  | ≡N                               |  |  |

**Legend:**

- ) the substance contained no functional groups
- 0) the substance did not adsorb antibodies
- a) adsorbent
- b) functional group
- c) quantity of AE of typhoid N-agglutinins adsorbed per 100 mg of adsorbent
- d) unadulterated agglutinating typhoid serum, titer: 25600 AE
- e) typhoid agglutinating serum submitted to adsorption by heterologous antigens, titer: 6400 AE
- f) naphthalene
- g) anthracene
- h) alizarin
- i) anthragallol
- j) indanthrene
- k) hexachlorocyclohexane
- l) benzidine (basic)
- m) ion exchange resin, AN-1
- n) greenish-blue phthalocyaninic pigment (blue G heliogen)

Bidistilled water and 0.85% sodium chloride solution was used as the dispersion medium to investigate the stability of suspensions of organic agents. The suspension was prepared by placing 50 mg of pulverized agent in a mortar, it was then ground, adding 5 ml [milliliters] of bidistilled water drop by drop. The suspension obtained was transferred to test tubes, it was left to stand for 5 to 20 minutes so that

the large particles could settle, and the supernatant suspension was then suctioned off. The obtained suspension contained particles 0.5 to 40 microns in size. Similarly suspensions were also prepared in 0.85% sodium chloride solution.



Electrophoregrams of typhoid agglutinogenic serum

Legend:

- I) adsorbed by heterologous antigens and twice filtered through asbestos sterilizing plates
- II) unadsorbed

The following method was used to study the stability of suspensions of organic agents following adsorption on particles of immune antibodies. Two methods were used to prepare the suspensions and achieve adsorption on antibody particles. Method I (published in Zhurnal mikrobiologii, epidemiologii i immunobiologii, 1961) was used in experiments with agents suspensions of which were stable in bidistilled water. Method II consisted of the following: into a mortar we placed 2.5 ml of agglutinogenic serum (1:5 dilution with a solution containing 0.85% sodium chloride and 1% boric acid) and 50 mg of pulverized agent being studied, the mixtures were ground until fine suspensions were produced and they were maintained for two hours at 37° after which we added 4 ml of 0.85% solution of sodium chloride and the suspension was then stored in a refrigerator at 6-8° for 18 hours; after 18 hours the suspensions were slowly centrifuged to remove large particles, the supernatant containing fine particles was suctioned off and again centrifuged rapidly; it was then decanted, and the precipitate was suspended, depending on the cloudiness of the suspensions, in 2 to 5 ml of 0.85% sodium chloride solution with a pH of 7.0. The stability of the suspensions following adsorption on antibody particles as well as their sensitivity and specificity under the influence of bacterial antigens were determined by the Adamov method (1960).

Suspensions that were stable in bidistilled water [doubly distilled] were obtained from 2-oxyanthraquinone, 1,2-dioxyanthraquinone, anthragallol and indanthrene power (Table 2). Following adsorption of antibodies (from sera adsorbed by heterologous antigens) by method I the alizarin, anthragallol and indanthrene suspensions remained stable, by method II stability was retained by alizarin, quinineizarin, anthragallol, indanthrene and

Table 2  
Relation of stability of suspensions of various agents to  
their chemical structure


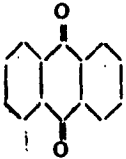
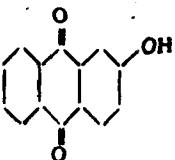
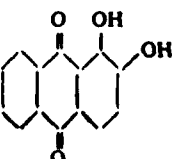
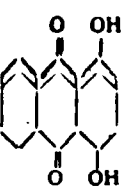
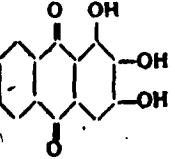
| Исследуемое вещество<br>(a)                   | Структурная формула<br>(b)  | Устойчивость частиц<br>(c)            |   | Устойчивость частиц<br>в 0,5% растворе по-<br>сле 24 часов хранения<br>по способу (e) |      |
|---|---|---------------------------------------|---|---|------|
|   |   | в бидистил-<br>лированной<br>воде (d) | в 0,5%<br>растворе<br>покрытого<br>сала (d) | I   | II   |
| (a) Антрацен                                  |    | ++++                                  | ++++  | 0   | ++++ |
| (b) Антрахинон                                |    | ++++                                  | ++++  | 0   | ++++ |
| (i) 2-оксидантрахи-<br>нон                    |   | -                                     | +++   | ++++  | ++++ |
| (d) Алizarин (1,2-<br>диоксидантрахи-<br>нон) |  | -                                     | ++++  | -   | -    |
| (k) Хинizarин                                 |  | ++++                                  | ++++  |   | ±    |
| (l) Антрагалдегид                             |  | -                                     | -   | -   | -    |

Table 2 [continued]

| (a)<br>Исследованное<br>вещество | (b)<br>Структурная формула | (c)<br>Устойчивость частиц                    |  | Устойчивость частиц<br>в 0,85% растворе по-<br>варенной соли после<br>адсорбции на них<br>высушенных антиде-<br>лов по способу (f) |     |
|----------------------------------|----------------------------|---|--|--|-----|
|                                  |                            | (d)<br>в би-<br>дистил-<br>лированной<br>воде | (e)<br>в 0,85%<br>растворе<br>поварен-<br>ной соли | I  | II  |
| Пурпурин<br>(m)                  |                            | ++++  | ++++   | 0  | +++ |
| Индантрон<br>(n)                 |                            | -   | ++++   | -  | -   |

## Legend:

- |  |                                       |
|--|---------------------------------------|
| a) agent studied   | g) anthracene                         |
| b) structural formula  | h) anthraquinone                      |
| c) particle stability  | i) 2-oxyanthraquinone                 |
| d) in bidistilled water  | j) alizarin (1,2-dioxyanthraquinone)  |
| e) in 0.85% table salt solution  | k) quinizarin                         |
| f) stability of particles in 0.85% salt solution following adsorption on them of immune antibodies using method: | l) anthragallol                       |
|  | m) purpurin [trihydroxyanthraquinone] |
|  | n) indanthrene [indanthrone?]         |

0) impossible to prepare suspension by this method

-) no agglomeration

+ to ++++) varying intensity of agglomeration

greenish-blue phthalocyaninic pigment containing no metal atoms. In several anthracene derivatives we observed a relation between suspension stability and chemical structure of the substances. Thus, suspensions of anthracene and anthraquinone were unstable in bidistilled water; introduction of a hydroxyl group to the anthraquinone molecule gave it



stability to particles of this substance (2-oxyanthraquinone) only in bidistilled water; addition of 2 hydroxyl groups to the first and second atoms of carbon of the anthraquinone molecule (alizarin substance) made the particles of this substance capable of producing stable suspensions in bidistilled water and in 0.85% sodium chloride solution following adsorption of antibodies on them. Introduction of two hydroxyl groups into the anthraquinone molecule, in the first and fourth atoms of carbon (quinizarin substance) made the particles stable in 0.85% salt solution only after antibodies were adsorbed on them.

Attachment of hydroxyl groups to the first, second and third atoms of carbon in the anthraquinone ring (anthragallol substance) made the particles of this substance stable in bidistilled water and in 0.85% salt solution. The anthragallol suspension remained stable even after adsorption on its particles of antibodies by methods I and II. Changing the position of one hydroxyl group, moving it from the third to the fourth atom (purpurin) made the purpurin particles unstable in both bidistilled water and 0.85% salt solution.

In addition to the above substances, the following also retained stability following antibody adsorption: particles of thionine, blue phthalocyaninic and green pigment.

Following antibody adsorption suspensions of alizarin, anthragallol, indanthrene, blue phthalocyaninic pigment, greenish-blue phthalocyaninic pigment and green pigment retained stability when they were mixed with antigens heterologous in relation to the antibodies adsorbed on the particles (Table 3). Suspensions of thionine and quinizarin, when mixed with antigens heterologous to antibodies adsorbed on these particles agglomerated with varying intensity depending on the form of antigen. With antigens that were homologous in relation to the antibodies adsorbed on suspension particles the highest immunological activity was demonstrated by antibodies adsorbed on alizarin and anthragallol. Agglomeration of these suspensions occurred with suspensions of *Salmonella typhosa* containing ten million or more cells per milliliter. Suspensions of other substances (indanthrene, blue phthalocyaninic and greenish-blue phthalocyaninic pigment, and green pigment) reacted only with suspensions of this pathogen containing 25 million bacterial cells per milliliter.

#### Conclusions

1. Adsorption of antibodies from immune sera was related to the chemical structure of sorbents and protein fraction ratio in the sera.
2. The stability of suspensions of particles of anthraquinone derivatives was related to the number of hydroxyl groups and their spatial arrangement.
3. The antibodies that were adsorbed on solid substances containing hydroxyl, imine and amine groups retained their immunological activity and

**Table 3**  
**Immunological activity and specificity of typhoid agglutinins**  
**adsorbed on particles of suspensions of different substances**

| (a)<br>Суспензия вещества, со-<br>держащая адсорбиро-<br>ванные брюшнотифозные<br>агглютинины | (b) Реакция агломерации с микробными антигенами |                                    |               |   |                                    |               |  |                       |
|---|---|------------------------------------|---------------|---|------------------------------------|---------------|--|-----------------------|
|   | (c) антигены брюшнотифоз-<br>ной палочки        |                                    |               | (d) антигены микробов<br>Флекснера (подтип 2a)  |                                    |               | взвесь ки-<br>шечной<br>палочки<br>(1 млрд.<br>в 1 мл) | 0.85% NaCl<br>раствор |
|   | (e)<br>микробная<br>взвесь<br>1:1000            | (f)<br>полный<br>антиген<br>1:1000 | (g)<br>гаптен | (h)<br>микробная<br>взвесь<br>1 млрд. в<br>1 мл | (i)<br>полный<br>антиген<br>1:1000 | (j)<br>гаптен |  |                       |
|   |   |                                    |               |   |                                    |               |  |                       |
| (k) Ализарин  | 10  | ++++                               | ++++          | -   | -                                  | -             | -  | -                     |
| (l) Антрагаллол   | 10  | ++++                               | ++++          | -   | -                                  | -             | -  | -                     |
| (m) Кинизарин   | 25  | ++++                               | ++++          | ++  | ++                                 | +             | ++   | ++                    |
| (n) Индантрон   | 25  | ++++                               | ++++          | ++  | ++                                 | +             | ++   | ++                    |
| (o) Тионин  | 25  | ++++                               | ++++          | ++++  | ++++                               | ++            | ++++   | ++                    |
| (p) Пигмент голубой фта-<br>лоцианиновый  | 25  | ++++                               | ++++          | ±   | ±                                  | ±             | ±  | ±                     |
| (q) Пигмент зеленовато-<br>голубой фталоциани-<br>новый (гелиоген го-<br>лубой Г)             | 25  | ++++                               | ++++          | ±   | ±                                  | ±             | ±  | ±                     |
| (r) Пигмент зеленый   | 25  | ++++                               | ++++          | ±   | ±                                  | ±             | ±  | ±                     |

**Legend:**

- a) suspension containing adsorbed typhoid agglutinins
- b) reaction of agglomeration with bacterial antigens
- c) *Salmonella typhosa* antigens
- d) Flexner's bacillus antigens (subtype 2a)
- e) bacterial suspension
- f) complete antigen
- g) haptene
- i) *E. coli* suspension (one billion per milliliter)
- j) 0.85% sodium chloride solution
- k) alizarin
- l) anthragalloi
- m) quinizarin
- n) indanthrene
- o) thionine
- p) blue phthalocyaninic pigment
- q) greenish-blue phthalocyaninic pigment (blue G heliogen)
- r) green pigment

Numerals indicate the minimal quantities of *Salmonella typhosa* inducing  
 ++ intensity of agglomeration.

-) absence of agglomeration

+ to ++++) varying intensity of agglomeration

engaged in specific reactions with homologous antigens.

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